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Bronchial hyperreactivity is associated with enhanced grain dust-induced airflow obstruction

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Kline, Joel N., Paul J. Jagielo, Janet L. Watt, and David A. Schwartz. Bronchial hyperreactivity is associated with enhanced grain dust-induced airflow obstruction. *J Appl Physiol* 89: 1172–1178, 2000.—Bronchial hyperreactivity (BHR) is associated with the presence of airway inflammation in asthma and is seen in individuals occupationally exposed to grain dust. To better understand the relationship between BHR and pulmonary inflammation after grain dust exposure, we carried out an inhalation challenge to corn dust extract (CDE) on seven subjects with BHR [a 20% or greater decrease in forced expiratory volume in 1 s (FEV_1) compared with diluent FEV_1 with a cumulative dose of histamine ≤47.3 breath units] and compared their physiological and inflammatory responses with those of seven matched control subjects. BHR subjects were exposed to nebulized CDE (target dose of 0.16 μg/kg endotoxin) as tolerated; matched controls received equal amounts of CDE. Subjects with BHR complained of chest tightness and dyspnea within the 2 h after inhalation of CDE significantly more frequently than controls. Similarly, subjects with BHR developed significantly greater percent declines in FEV_1 at time points up to 4 h after exposure to CDE. Significant increases in total cells, neutrophils, tumor necrosis factor-α, interleukin-6, and interleukin-8 were detected in bronchoalveolar lavage fluid 4 h after inhalation of CDE in all subjects, but no differences were detected between the control and BHR groups. These results suggest that, although subjects with BHR develop a more precipitous decline in FEV_1 after exposure to CDE, the inflammatory response to CDE is similar in subjects with and without BHR.

inhalation exposure; airway inflammation; endotoxin; lipopolysaccharide

EPIDEMIOLOGICAL STUDIES OF GRAIN WORKERS have demonstrated an excess of respiratory symptoms and airflow obstruction associated with chronic exposure to grain dust (6, 7, 12–14). Studies looking at specific host factors, such as atopy or the presence of specific antibodies to grain dust, have not found them to be consistently associated with either acute (11) or chronic (31) airway responses to grain dust. Other host factors, such as smoking, age, and duration of employment, have been associated with greater longitudinal declines in lung function (31). In addition, it appears that acute changes in airflow over a work shift or workweek are predictive of accelerated longitudinal declines in airflow (7, 20, 31). In fact, in grain workers with nonspecific bronchial hyperreactivity (BHR), there is an association between work-shift changes in forced expiratory volume in 1 s (FEV_1) and longitudinal declines in FEV_1, whereas no association was seen in workers with normal airway reactivity (15). These findings would suggest that BHR might be an important host factor contributing to the pathogenesis of chronic airflow limitation due to grain dust.

Airway inflammation appears to be essential to the development of grain dust-induced airflow obstruction. Our laboratory previously demonstrated that the endotoxin content of grain dust is an important determinant of the development (30) and progression (28) of airway disease among exposed workers and of the ability of grain dust to induce airflow obstruction and inflammatory responses in the airway (18, 19, 29). Inhaled endotoxin can induce airflow obstruction in naive or previously unexposed subjects, as well as those chronically exposed (9). Indeed, even among normal, nonatopic, nonasthmatic, nonsmoking subjects, some individuals exhibit a hypersensitive bronchospastic response after the inhalation of endotoxin (21).

The inflammatory response to inhaled grain dust is characterized by an exuberant chemotaxis of alveolar macrophages and neutrophils to the airways and alveolar spaces (8–10, 19, 34). Grain dust exhibits direct chemotactic activity for neutrophils (36) and induces the release of interleukin (IL)-1β (22) and other factors such as tumor necrosis factor-α (TNF-α), IL-6, and neuropeptides (33). Inhalation studies in humans (8–10, 18, 35) and mice (10, 18, 29) have shown that, after a single inhalation challenge with grain dust, neutrophils are recruited to the lung and that proinflammatory cytokines (IL-1β, TNF-α, and IL-6) and chemokines (IL-8 and macrophage inflammatory protein-2) are produced and released for up to 48 h (10). These mediators are actively synthesized by macrophages and neutrophils (37). Thus induction of inflammatory cells such as alveolar macrophages and neutrophils are central to the response that follows inhalation of grain dust; these cells are associated with expression of mul-

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tiple inflammatory mediators that are likely to be redundant and amplifying in effect.

The purpose of the present investigation is to further investigate the role of BHR as it relates to the acute physiological and inflammatory events due to acute grain dust inhalation. By using an acute-exposure model of grain dust-induced airway inflammation, our goal was to compare the acute physiological and inflammatory changes after exposure to grain dust in subjects with and without BHR. Our hypothesis was that both the physiological and inflammatory changes after exposure to grain dust were more pronounced in subjects with BHR and that airway inflammation would be associated with the development of airflow obstruction.

METHODS

We used a single-blind, crossover design in subjects with and without BHR to determine whether bronchial hyperreactivity affected the acute physiological and inflammatory changes after acute inhalation of corn dust extract (CDE). All experimental protocols and consent forms were reviewed and approved by the Institutional Review Board (Human Subjects Review, Committee A) of the University of Iowa.

Study subjects. Subjects who were healthy, had never smoked, and were without any history of prior cardiac disease or occupational exposure to grain dust were recruited. Advertising requested nonsmoking subjects with no known lung disease or subjects with occasional respiratory symptoms. To be considered eligible for participation, all study subjects were required to have a normal physical examination, 12-lead electrocardiogram, chest X-ray, and pulmonary function tests (spirometry, lung volumes, diffusing capacity, and arterial blood gases). A standard histamine challenge test was performed on each subject, which included five inhalations of 0.03, 0.06, 0.12, 0.25, 0.5, 1.0, 2.5, 5.0, and 10.0 mg/ml concentrations of buffered histamine at room temperature, delivered according to the guidelines established by the American Academy of Allergy, Committee on Standardization of Bronchoprovocation (4). The cumulative dose (in breath units) of histamine causing a 20% fall in baseline FEV_1 compared with diluent (sterile isotonic saline solution) or up to a maximum dose of 97.3 breath units was determined. Bronchial hyperreactivity was defined as a 20% or greater decrease in FEV_1 compared with diluent FEV_1 with a cumulative dose of histamine ≤47.3 breath units. The slope of the dose-response curve was calculated by dividing the maximal percent drop in FEV_1 by the cumulative breath units causing this decline (27). Individuals in the study who were screened and found to have BHR were limited to subjects who were never previously diagnosed with asthma or who had a history of stable, mild, intermittent asthma with only occasional (less than twice per week) use of inhaled β-agonists. Subjects who were taking antihistamines, theophylline, inhaled corticosteroids, or other chronic medications were excluded from participation. All subjects were screened for atopy by using a standard panel of aeroallergens and were nonatopic. Subjects on inhaled β-agonists were instructed to discontinue the drug for 24 h before both the histamine challenge and each inhalation exposure. Subjects with BHR were matched with subjects demonstrating normal airway reactivity and of similar age (within 5 yr), gender, and body height (within 5 cm) and weight (within 5 kg).

Protocol. All study subjects underwent two separate inhalation challenges (saline and CDE), with exposures separated by at least 2 wk. Previously, our laboratory demonstrated that lung function and lavage parameters return to baseline values within 48–96 h after inhalation of grain dust (10). To ensure continued participation in this trial, all subjects were exposed to saline on the first visit and CDE on the second visit, although the subjects were not informed about the order of the exposures. Vital signs, pulmonary function, and symptomatology were recorded before and after each inhalation exposure by using an established protocol.

Preparation of the CDE. Corn dust used in this study was obtained from the air-filtration system at an eastern Iowa grain facility. CDE was prepared by mixing 3.0 g of dust in 30 ml of sterile, pyrogen-free Hanks' balanced saline solution (HBSS) without calcium or magnesium (0.1% solution), vortexing for 2 min, and shaking for 1 h at 4°C. The mixture was centrifuged at 800 *g* for 20 min, and the supernatant solution was collected, resulting in the CDE. The CDE solution underwent filter sterilization through a 0.22-μm filter (Acrocap Low Protein Binding Filter, Gelman Sciences, Ann Arbor, MI). All solutions used for inhalation were derived from a stock solution that underwent sterility testing (bacteria and fungi) and endotoxin assay before separation into individual aliquots. These aliquots were stored at −70°C before use. Although levels of mycotoxins, such as aflatoxin and fumonisin, were not measured in these aliquots, only negligible concentrations have been previously detected in similar samples. Endotoxin concentration was measured by the endpoint chromogenic *Limulus* amebocyte lysate assay (QCL-1000, Whittaker Bioproducts, Walkersville, MD). The measured endotoxin concentration in the CDE prepared by this method was 4.0 μg/ml.

Inhalational challenge. The solutions were administered via a nebulizer (model 646, DeVilbiss, Somerset, PA) and dosimeter (DeVilbiss), operated at 20 psi air pressure. Subjects, who were in the seated position during exposure and subsequent pulmonary function testing, controlled the timing of each nebulized dose and were instructed to inhale through the mouthpiece of the nebulizer and exhale through their nose. By using this delivery system and technique, a precise dose of inhalant was delivered. For each exposure, the goal was to administer 0.04 ml of inhalant (CDE or HBSS) per kilogram of body weight [or 0.16 μg lipopolysaccharide (LPS)/kg] by using continuous tidal respirations over a 60-min period of time. This dose of LPS was previously identified as equivalent to an average work-day inhalation exposure to LPS for a grain elevator worker (8, 9). Three of seven of the CDE inhalational challenges (but none of the saline exposures) to BHR subjects were terminated as a result of complaints by the subjects of severe chest tightness, dyspnea, or cough. Matched control subjects without BHR were then given equal amounts of CDE as the BHR subjects.

Pulmonary function testing. The pulmonary function tests consisted of serial measurements of airflow by a spirometer (Spirotech S-600, Graseby Anderson, Atlanta, GA). These maneuvers were performed by using standard protocols and American Thoracic Society guidelines (2). The spirometer was calibrated before each visit. With the subjects wearing nose clips and in a sitting position, spirometry was performed preexposure and at the following time points postexposure: 10, 20, and 30 min, and 1, 2, 3, 4, and 24 h.

Bronchoscopy. Bronchoscopy was performed 4 h after each inhalation exposure, in accordance with the standards established by the American Thoracic Society for bronchoscopy in asthmatic subjects (3). This time point was chosen because of previous studies in which airway inflammatory responses were assessed by bronchoscopy after exposure to grain dust extracts (8, 10). Subjects were pretreated with atropine in-

jection and inhaled bronchodilators (albuterol metered dose inhaler). Supplemental low-flow (3 l/min) oxygen was administered during the procedure, and no subjects suffered oxygen desaturation. An Olympus P-10 (Lombard, IL) fiber-optic bronchoscope was introduced transorally into the chosen lung segment for bronchoalveolar lavage (BAL) and wedged. Twenty milliliters of sterile, 0.9% saline (37°C) were injected through the bronchoscope and then collected. This procedure was performed five more times for a total lavage volume of 120 ml. The return of the first 20 ml of aliquot was separated from the remaining lavage fluid and discarded. Lung segments chosen for BAL alternated between a subsegment of the right middle lobe after the first exposure and a subsegment of the lingula after the second exposure.

Processing of specimens. Immediately after bronchoscopy, the BAL samples were processed according to methods described previously (8). The BAL supernatant was frozen at −70°C for subsequent use. After the cells were washed twice with HBSS, the cell pellet was suspended in RPMI 1640 medium and cell counts were performed. Cytospin preparations were made from the lavage cell resuspension and stained with Diff Quick staining (Baxter Scientific Products, Miami, FL), and cell differential counts were quantified by counting 200 cells. TNF-α, IL-6, and IL-8 were measured in the BAL supernatant fluid by using commercially available enzyme immunoassays (R&D Systems, Minneapolis, MN).

Statistics. CDE-induced changes in lung function and biological measures of inflammation (BAL cellularity and BAL and cytokines) were performed by comparing normal and BHR subjects with the use of nonparametric paired statistics (Wilcoxon rank-sum test, 2-tailed) (26). Comparison of symptom frequency was performed by using Fisher's exact test. A value $P < 0.05$ was considered significant.

RESULTS

Baseline comparison of subjects with BHR and control subjects. A total of 14 subjects participated in and completed the study (12 women, 2 men). The BHR subjects were matched by gender, age, weight, and height to control subjects without reactive airways (Table 1). As expected, there was a significant difference in both the slope of the dose-response curve to histamine between the control and BHR groups (Table 1) and the baseline FEV_1-to-forced vital capacity ratio (FEV_1/FVC) (Table 2) but not in any other measured pulmonary function parameter (Table 2).

Of the seven subjects with BHR, only four subjects were able to inhale the full intended dose (0.16 μg/kg endotoxin) of CDE. One subject developed bronchospasm after exposure to less than one-half of the calculated dose of CDE. A second subject with BHR received 33%, and another subject with BHR received 90% of the calculated dose, at which time they were unwilling to complete the exposure because of intolerable symptoms of chest tightness. The matched control subjects were given equal doses of CDE as the proband subjects with BHR. Although these control subjects without BHR received equivalent doses of CDE as the BHR subjects, none complained of symptoms requiring cessation of the protocol.

Table 1. *Demographics*

	Control Subjects	BHR Subjects	*P* Value
Age, yr	26.6 ± 2.7	24.8 ± 1.9	NS
Gender, female/male	6/1	6/1	NS
Weight, kg	72.4 ± 6.4	74.4 ± 5.7	NS
Height, cm	167.5 ± 2.6	169 ± 4.2	NS
Histamine slope*	0.05 ± 0.017	5.15 ± 2.39	<0.005

Values are means ± SD. BHR, bronchial hyperreactivity; NS, not significant. *Slope of the histamine dose-response curve [%change in forced expiratory volume in 1 s (FEV_1)/breath units histamine].

Table 2. *Baseline pulmonary function*

	Control Subjects	BHR Subjects	*P* Value
FEV_1, liters (1st s)	3.28 ± 0.14(97)	3.21 ± 0.28(93)	NS
FVC, liters	4.02 ± 0.17(94)	4.43 ± 0.51(100)	NS
FEV_1/FVC	0.83 ± 0.09	0.74 ± 0.06	<0.05
SVC, liters	4.05 ± 0.18(95)	4.59 ± 0.60(105)	NS
RV, liters	1.55 ± 0.13(91)	1.44 ± 0.22(85)	NS
TLC, liters	5.61 ± 0.24(98)	6.03 ± 0.65(104)	NS
DL_{CO}, ml CO·min^{-1}·mmHg^{-1}	31.8 ± 2.76(137)	31.6 ± 4.10 31.7(134)	NS

Values are means ± SD with % predicted in parentheses. FVC, forced vital capacity; SVC, slow vital capacity; RV, residual volume; TLC, total lung capacity; DL_{CO}; diffusion capacity for carbon monoxide.

Symptomatic response to inhaled CDE. Respiratory and nonrespiratory symptoms were reported by subjects after exposure to CDE, including chest tightness, dyspnea, cough, sputum production, malaise, and chills. None of these symptoms was reported after inhalation of HBSS. When the frequency of these symptoms was compared in subjects with and without BHR, only chest tightness and dyspnea were found to be significantly different between these groups (Table 3). In subjects with BHR, chest tightness was experienced by a majority of the participants for at least the first 2 h postexposure, with subsequent decline. Only one control subject experienced chest tightness lasting more than 10 min. Similarly, four subjects with BHR experienced dyspnea lasting at least 1 h after inhalation of CDE, whereas no control subjects complained of dyspnea. There were no significant differences in the number of subjects reporting cough, chills, sputum

Table 3. *Symptoms*

	Control Subjects	BHR Subjects	*P* Value
Chest tightness			
10 min	2	5	NS
20 min	1	5	<0.05
30 min	1	5	<0.05
1 h	1	6	<0.05
2 h	1	5	<0.05
3 h	1	3	NS
4 h	1	3	NS
Dyspnea			
10 min	0	4	<0.05
20 min	0	4	<0.05
30 min	0	4	<0.05
1 h	0	4	<0.05
2 h	0	1	NS
3 h	0	1	NS
4 h	0	1	NS

Values given as no. of subjects with complaint.

production, or malaise at each of the time points queried (data not presented).

Pulmonary physiological response to inhaled CDE. Acute airflow obstruction developed after exposure to CDE (but not after exposure to HBSS) in subjects both with and without BHR, occurring as early as 10 min postexposure and persisting for at least 4 h postexposure. This was demonstrated by declines in FEV_1 (Fig. 1) and in FEV_1/FVC (data not shown). Although both groups developed abrupt declines in FEV_1 within 10 min after inhalation of CDE, the BHR group had significantly greater declines in both FEV_1 and FEV_1/FVC. At 10 min postexposure, the mean percent decline in FEV_1 from baseline in subjects with BHR was 42%, which was significantly greater than control subjects (11%; $P < 0.01$). Over the first 2 h after exposure to CDE, subjects with BHR continued to have significantly greater declines in FEV_1 compared with subjects with normal airway reactivity, although the magnitude of difference declined over time as a result of gradual improvement in FEV_1 in the BHR subjects. Interestingly, the greater percent decline in FEV_1 seen in the BHR group was associated with increased subjective reporting of chest tightness and dyspnea (Table 3).

Inflammatory response to inhaled CDE. An acute inflammatory response in the lower respiratory tract was observed after exposure to CDE compared with saline for normal control subjects as well as those with BHR (Fig. 2). The inflammatory response consisted predominately of increases in concentrations of total cells and neutrophils. Although these BAL cell concentrations increased significantly after inhalation challenge with CDE in both normal subjects and those with BHR, no differences were seen between these groups (Fig. 2).

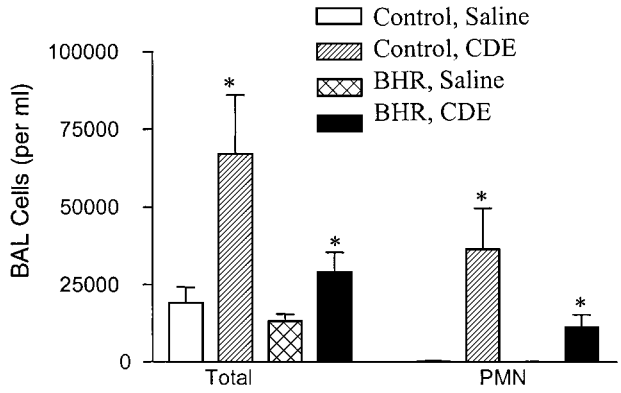

Fig. 2. Change in bronchoalveolar lavage (BAL) cellularity after inhalation challenge. After an inhalation challenge with saline or CDE, normal control subjects and subjects with BHR underwent BAL. Total numbers of cells and the percentage of polymorphonuclear cells (PMN) in the BAL fluid were significantly increased in both normal control and BHR subjects after exposure to CDE compared with saline. There were no significant differences in BAL cellularity between normal control and BHR subjects. $*P < 0.01$, saline vs. CDE exposure.

In subjects with and without BHR, exposure to CDE (in comparison to saline) resulted in significant increases in the concentration of BAL fluid TNF-α, IL-6, and IL-8 (Fig. 3). However, post-CDE concentrations of TNF-α, IL-6, and IL-8 did not significantly differ between subjects with and without BHR.

DISCUSSION

Our results indicate that subjects with BHR develop greater respiratory symptoms and airflow obstruction after inhalation of CDE compared with subjects with no evidence of airway hyperreactivity. The initial marked decline in airflow obstruction appears to slowly

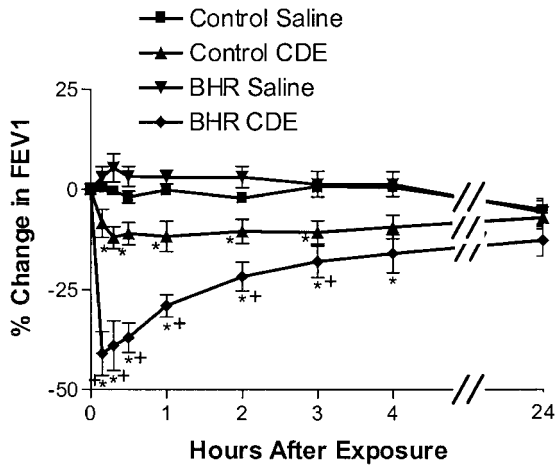

Fig. 1. Percent change in forced expiratory volume in 1 s (FEV_1) after inhalation challenge. Subjects with bronchial hyperreactivity (BHR) and control subjects underwent baseline spirometry followed by a saline inhalation challenge. Spirometry was subsequently repeated at various time points. At least 2 wk later, a corn dust extract (CDE) challenge was performed, followed by spirometric monitoring. Both control subjects and those with BHR developed significant reductions in their FEV_1 after exposure to CDE but not to saline. The reduction was significantly greater in the BHR group than the control group. $*P < 0.01$, CDE vs. saline. $^{+}P < 0.01$ BHR vs. control.

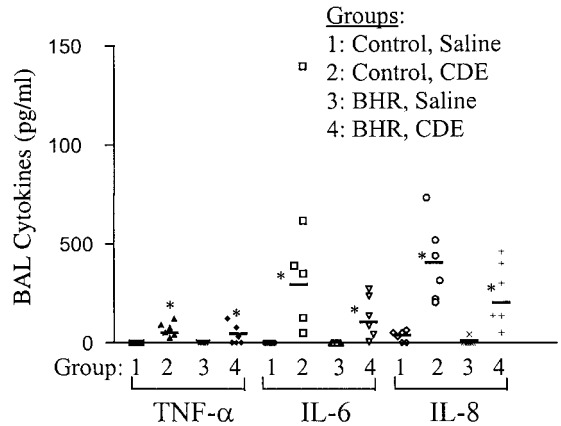

Fig. 3. Change in BAL cytokines after inhalation challenge. After an inhalation challenge with saline or CDE, control subjects and subjects with BHR underwent BAL. Concentrations of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8) were significantly increased in both control and BHR subjects after exposure to CDE. No significant differences were noted between control and BHR subjects. Symbols represent different subjects. $*P < 0.01$, saline vs. CDE exposure.

improve over the first 4 h after inhalation exposure, resulting in a similar pattern (though of greater magnitude) of airflow reduction as that observed in normal subjects. In contrast to the distinct physiological differences observed between subjects with and without BHR, subjects with BHR do not demonstrate a greater inflammatory response to CDE as measured by BAL cellularity and cytokine concentrations. Our findings indicate that individuals with airway hyperreactivity are more responsive to the bronchoconstrictive effects of inhaled CDE and provide support for the hypothesis that BHR and airway inflammation are incompletely linked phenomena in airway diseases such as asthma.

Previous studies have examined the response of asthmatic individuals to inhaled endotoxin (23–25). Michel and colleagues found that inhalation of 22 μg of LPS induced a small reduction in FEV_1 in asthmatic but not in normal individuals (23) that was associated with increased nonspecific BHR (25). Our present study bolsters these studies by demonstrating a significantly greater degree of airflow obstruction after inhalation of CDE by subjects with BHR than was seen in normal control subjects. These data support the proposal that asthmatic individuals and those with BHR are more likely to develop symptomatic airflow obstruction when exposed to dusts containing high levels of endotoxin. These findings may explain why individuals with BHR develop more progressive airway disease when working with grain dust (7).

The mechanism by which CDE produces an initial exaggerated physiological response in subjects with BHR was not explored in this study, but it is clearly of interest. Extracts of grain dust have been shown to cause the release of histamine and leukotrienes from human lung tissue (5). Similarly, endotoxin, a major component of grain dust, may cause the release of preformed mediators such as histamine (32), resulting in bronchoconstriction. These substances may cause rapid, short-term declines in airflow that may be exaggerated in subjects with underlying BHR. Alternatively, inhalation of CDE may cause acute bronchoconstriction through neurally mediated mechanisms, such as through cholinergic pathways or nonadrenergic, noncholinergic neuropeptide mediators. However, previously, our laboratory was not able to demonstrate detectable levels of histamine, 15-hydroxyeicosatetraenoic acid, PGE_2, or leukotriene B_4 in BAL fluid of normal control subjects 4 h after exposure to CDE (8).

More surprising than our finding of increased induction of airflow obstruction in subjects with BHR was that the pulmonary inflammatory responses were not different between subjects with and without BHR. In an earlier study, Michel et al. (24) found a small but significant increase in the concentration of plasma TNF-α, peripheral leukocytosis, and neutrophils among asthmatic subjects after inhalation of LPS. This present study differs from previous studies in that the protocol (delivered as CDE) resulted in delivery of a significantly lower amount of inhaled endotoxin to the subjects. The subjects were then evaluated by bronchoscopy, a more specific measure of the airway inflammatory response than measures of blood parameters. Although both normal subjects and those with BHR developed substantial airway inflammation after inhalation of CDE, there were no significant differences in these inflammatory responses between the two groups. There are a number of potential explanations for the similar levels of inflammatory cells and mediators in the BAL fluid obtained from the two groups after CDE exposure. First, the lavage concentrations of cells and cytokines are relatively crude indicators of airway inflammation in the region most pertinent to asthma. Indeed, the BAL sample is more representative of distal alveolar processes than the more proximal small airways. Second, the cellular and protein mediators of inflammation that we chose to measure, on the basis of previous studies demonstrating their induction by endotoxin and by grain dust (8–10), may not be the mediators most relevant to the expression of bronchospasm. Alternative mediators may include neuropeptides, such as substance P, that are induced in a hamster model by grain dust (16, 17) and blocked by the anti-inflammatory agent dexamethasone (1). A potentially more provocative explanation for the lack of difference in induction of inflammation in subjects with and without BHR is that airflow obstruction may actually provide protection from environmental stimuli. Although we did not measure FEV_1 throughout the exposure period, it is likely that reductions in FEV_1 were occurring during the period of inhalation challenge, as shown in nonasthmatic individuals in previous studies (21). This decrease in airflow may have altered the distribution of aerosol in the lung, preventing aerosol from being deposited in the distal regions of the lung in subjects with BHR. Thus BHR may act to protect individuals from environmental exposures, such as grain dust, by reducing the overall exposure, resulting in less inflammation in the lower respiratory tract. In contrast, subjects with nonreactive airways may be more likely to tolerate these exposures for longer periods of time, but, as a consequence, they develop greater airway inflammation in the lower respiratory tract. Finally, the genetics of BHR (in this study, as defined by sensitivity to inhaled histamine) may differ from the genetics of the inflammatory response to inhaled endotoxin. Our laboratory recently demonstrated that both the inflammatory response and the bronchospastic response to inhaled endotoxin vary widely in normal, nonasthmatic subjects (21). The inflammatory response to inhaled endotoxin may be unrelated to BHR.

In conclusion, it appears that BHR is a major host factor that is associated with exaggerated initial declines in airflow after acute grain dust exposure but may be protective in reducing the magnitude of the acute inflammatory cell recruitment in the lower respiratory tract. It is possible that the mechanism underlying BHR, in conjunction with repetitive bouts of bronchoconstriction and airway inflammation associated with chronic exposure to grain dust, may be responsible for producing the chronic, irreversible airflow obstruction. The significantly greater and more persis-

tent bronchospasm that follows inhalation of endotoxin-containing CDE by asthmatics may be responsible for the "healthy worker effect," in which disease-susceptible individuals leave the work force. An important future study suggested by these findings includes comparison of the bronchospastic and inflammatory responses of workers occupationally exposed to grain dust who do or do not develop significant symptomatology. These results suggest that differences in symptoms and in the development of bronchospasm may not be reflected in different levels of airway inflammation between those groups.

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